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Impact of three days high and low dietary sodium intake on sodium status in response to exertional-heat stress: A double-blind randomized control trial

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Abstract

Purpose: To determine the impact of altering dietary sodium intake for three days preceding exercise on sweat sodium concentration ($[Na^+]$), cardiovascular and thermoregulatory variables.

Methods: Fifteen male endurance athletes (runners n=8, cyclists n=7) consumed a low (LNa, 15mg·kg^{-1}·day^{-1}) or high (HNa, 100mg·kg^{-1}·day^{-1}) sodium diet, or their usual free-living diet (UDiet, 46 (37-56)mg·kg^{-1}·day^{-1}) for three days in a double-blind, randomized cross-over design, collecting excreted urine (UNa) and refraining from exercise. On day four they completed 2 h running at 55% $\dot{V}O_{2max}$ or cycling at 55% maximum aerobic power in T_{amb} 35^{o}C. Pre- and post-exercise blood samples were collected, and sweat from five sites using absorbent patches along the exercise protocol.

Results: UNa on days 2-3 pre-exercise (mean(95% CI): LNa 16(12-19)mg·kg^{-1}·day^{-1}, UDiet 46(37-56)mg·kg^{-1}·day^{-1}, HNa 79(72-85)mg·kg^{-1}·day^{-1}; $p<0.001$) and pre-exercise aldosterone (LNa 240(193-286)mg·kg^{-1}·day^{-1}, UDiet 170(116-224)mg·kg^{-1}·day^{-1}, HNa 141(111-171)mg·kg^{-1}·day^{-1}; $p=0.001$) reflected sodium intake as expected. Pre-exercise total body water was greater following HNa compared to LNa ($p<0.05$), but not UDiet. Estimated whole body sweat $[Na^+]$ following UDiet was 10-11% higher than LNa and 10-12% lower than HNa ($p<0.001$), and correlated with pre-exercise aldosterone (1st h r=-0.568, 2nd h r=-0.675; $p<0.01$). Rectal temperature rose more quickly in LNa *vs* HNa (40-70 min; $p<0.05$), but was similar at the conclusion of exercise, and no significant differences in heart rate or perceived exertion were observed.

Conclusions: Three days altered sodium intake influenced urinary sodium excretion and sweat $[Na^+]$, and the rise in rectal temperature, but had no effect on perceived exertion during moderate intensity exercise in hot ambient conditions.

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Keywords: Salt, Sweat, Endurance, Running, Cycling, Plasma volume, Plasma osmolality.

Abbreviations:

CHO – Carbohydrate

CI – Confidence interval

CV – Coefficient of variation

FA – Forearm

FH – Forehead

GIS – Gastrointestinal symptoms

Hb – Haemoglobin

HCT – Haematocrit

HNa – High sodium diet (100 $mg \cdot kg^{-1} \cdot day^{-1}$)

HR – Heart rate

ISE – Ion selective electrode

LNa – Low sodium diet (15 $mg \cdot kg^{-1} \cdot day^{-1}$)

LSR – Local sweat rate

MAP – Maximum aerobic power

MT – Mid-thigh

Na^+ – Sodium

$[Na^+]$ – Sodium concentration

NaCl – Sodium chloride

P_{Osm} – Plasma osmolality

P_v – Plasma volume

RPE – Rating of perceived exertion

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SD – Standard deviation

SS – Superior scapula

T_{amb} – Ambient temperature

TBW – Total body water

TCR – Thermal comfort rating

T_{re} – Rectal temperature

UC – Upper chest

UDiet – Usual free-living diet (mean: 46 $mg \cdot kg^{-1} \cdot day^{-1}$)

UNa – Urinary sodium excretion

USG – Urine specific gravity

$\dot{V}O_{2max}$ – Maximal oxygen uptake

$\dot{V}O_{2peak}$ – Peak oxygen uptake

WB – Whole body

WBW – Whole body washdown

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Introduction

During endurance exercise, metabolic heat production results in the production of sweat, in order to reduce body temperature through evaporation from the skin (Sawka et al., 2007). The composition of sweat includes significant quantities of sodium (Na^+), which is the most abundant cation, present in typical concentrations of 12 to 105 $mmol \cdot L^{-1}$ (Baker et al., 2016). As a result, endurance exercise, particularly in hot ambient conditions, can lead to substantial sodium losses, albeit proportionally less than water (Shirreffs & Sawka, 2011). This has placed much emphasis on the dietary sodium needs of endurance athletes by researchers (Baker et al., 2016; Shirreffs & Sawka, 2011), athletes and their support networks (McCubbin et al., 2018). However, to date there are no quantifiable guidelines for sodium intake before, during or after endurance and ultra-endurance exercise (Hoffman et al., 2019a; Thomas et al., 2016).

There are several factors that can influence the sodium concentration ($[Na^+]$) in sweat during exercise. The initial sweat produced by the secretory coil of the sweat gland is generally similar $[Na^+]$ to plasma (Sato et al., 1989). Therefore, factors that affect fluid balance, and hence plasma $[Na^+]$ both before and during exercise, are likely to influence sweat $[Na^+]$ (Morgan et al., 2004). Sweat composition is also altered through ion reabsorption in the reabsorptive duct as it travels towards the skin surface (Sato et al., 1989). The flow rate of sweat through the duct impacts on the ability for ion reabsorption, such that high flow rates, as occurs from increased rates of sweat production, reduce the completeness of reabsorption and result in higher sweat $[Na^+]$ (Buono et al., 2008). The rate of sweat production is influenced by multiple factors during exercise, including exercise intensity, ambient conditions, and airflow over the skin (Holmes et al., 2016; Saunders et al., 2005; Sawka et al., 2007). The reabsorptive capacity of the sweat gland is also at least partially regulated,

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with heat acclimation shown to significantly increase Na^+ reabsorption, resulting in lowered sweat [Na^+] (Chinevere et al., 2008).

Dietary sodium intake is also thought to influence the reabsorptive capacity of sweat glands, with McCance (1938) showing that inducing sodium deficiency through the combination of dietary restriction and sweating results in a progressive reduction in sweat [Na^+] to conserve total body sodium stores. However, this and other earlier studies primarily collected sweat samples during low intensity exercise or over both exercise and rest periods, in sedentary or untrained populations, and with sodium intakes that do not reflect those typical of endurance athletes (Conn et al., 1946; Ramanathan et al., 1956; Robinson et al., 1950, 1955). A recent study that surveyed endurance athletes found that 58% intended to either deliberately, or unintentionally through increased overall food intake, increase sodium consumption compared to their usual diet in the days preceding competition, and for a typical period of 2-5 days, whereas only 3% intended to reduce sodium intake (McCubbin et al., 2018). For researchers studying the effect of sweat sodium replacement during exercise, the impact of altered dietary sodium intake in the days preceding exercise also represents a potential confounding variable, in that attempts to replace a specific proportion of sweat sodium losses relies on accurate estimations of expected losses in the first place. For this to occur, the impact of dietary sodium intake on sweat [Na^+] must be understood, and controlled for if necessary.

The influence of dietary sodium intake on sweat [Na^+], in athletes and specifically during endurance exercise, remains unclear (McCubbin & Costa, 2018). The varied results reported between studies may be due to several factors, including poor validation of sodium balance in the days preceding exercise, collection of sweat samples from limited regional sites, averaging sweat [Na^+] data across multiple body sites or collection days, and insufficient

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137 statistical analysis or reporting (McCubbin & Costa, 2018). Furthermore, to date, no studies
138 have provided participants with dietary sodium proportional to body mass. Given that both
139 total body sodium stores (Kennedy et al., 1983) and total body water (TBW) (Watson et al.,
140 1980) are proportional to body mass, it would seem prudent for dietary sodium interventions
141 to follow this approach, to achieve consistency in whole body sodium balance between
142 participants.

143 Changes in dietary sodium intake preceding exercise may also influence cardiovascular
144 function and thermoregulation, through changes in plasma volume (P_v), plasma osmolality
145 (P_{Osm}), and plasma [Na^+] (Sims et al., 2007a,b; Hamouti et al., 2014; Armstrong et al., 1985;
146 Koenders et al., 2017). Sodium loading 2-3 h pre-exercise (38 $mg \cdot kg^{-1}$) has been shown to
147 expand P_v 2-5% when consumed with 10 $mL \cdot kg^{-1}$ water, with minimal or no change in P_{Osm}
148 (Sims et al., 2007a,b; Hamouti et al., 2014). However, few studies have altered sodium
149 intake for 1-5 days as frequently practiced by athletes (McCubbin et al., 2019), or have done
150 so during a simultaneous period of heat acclimation (Armstrong et al., 1985; Konikoff et al.,
151 1986), creating difficulties in their interpretation. An increased P_v prior to exercise in
152 response to increasing sodium intake, if maintained throughout the exercise bout, has
153 potential to increase stroke volume and cutaneous blood flow, resulting in reductions in
154 heart rate and core body temperature, respectively (Trangmar & González-Alonso, 2017).
155 Altered P_{Osm} may also influence sweat rate, independently of $P_{v.}$ (Takamata et al., 1995,
156 2001). However, to date the effect of P_{Osm} has not specifically been studied in an exercise
157 model of heat stress, using the 1-5 day timeframe in which athletes typically alter dietary
158 sodium intake.

159 The purpose of this study was therefore, to investigate the effect of three days of high and
160 low dietary sodium intakes, proportional to body mass, on sodium balance and associated

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variables (urinary sodium excretion (UNa), plasma and sweat [Na^+]) before and during endurance exercise in the heat). Additionally, to investigate the subsequent effects on heart rate, rectal temperature, thermal comfort and perceived exertion, and to compare these to the participant's usual free-living diet. We hypothesized that there would be a significant difference in UNa, plasma and sweat [Na^+] between dietary conditions, despite only a three-day dietary intervention, but minimal difference in hydration status, cardiovascular or thermoregulatory variables.

Methods

Ethical Approval

This study conformed to the standards set by the Declaration of Helsinki, and was approved by the Monash University Human Research Ethics Committee. All participants gave written informed consent prior to participating in the study.

Participants

Fifteen non heat-acclimatized, endurance-trained male runners, cyclists and triathletes volunteered to participate in this study (mean ± SD: age 40 ± 5 yr, height 179 ± 5 cm, body mass 77.1 ± 5.0 kg, body fat mass 17.1 ± 4.8%, training volume 8.6 ± 5.5 $h \cdot wk^{-1}$, $\dot{V}O_{2max}$ 55.6 ± 4.7 $mL \cdot kg^{-1} \cdot min^{-1}$). Participants were excluded if they had known Cystic Fibrosis, renal failure or other chronic conditions that impair kidney or sweat gland function, or musculoskeletal injury that would impair their ability to complete the required exercise task.

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Participants opted to either complete the experimental procedure cycling (n= 7) or running (n = 8), depending on their usual sporting participation and personal preference.

Preliminary Measures and Familiarization

Seven to fourteen days prior to the first experimental trial, participants attended the laboratory where height, nude body mass, fat and fat free mass were measured (Seca 515 MBCA; Seca Group, Hamburg, Germany). Maximum oxygen uptake ($\dot{V}O_{2max}$) was estimated by continuous incremental exercise test to volitional exhaustion (Vmax Encore Metabolic Cart; Carefusion, San Diego, Calif., USA), for runners on a motorized treadmill (Forma Run 500; Technogym, Seattle, WA, USA) as previously reported (Costa et al., 2009), and for cyclists on their own bicycle attached to a Wahoo KICKR cycle ergometer (Wahoo Fitness, Atlanta, GA, USA) previously validated in the power output range of all participants' maximum aerobic power (MAP) (Zadow et al., 2016), using an incremental protocol previously reported (Currell & Jeukendrup, 2008). Participants rode the Wahoo KICKR for approximately 10 min then performed a spin-down calibration prior to testing. Running speed for experimental trials was determined as the speed at 1% gradient that produced approximately 55% of $\dot{V}O_{2max,}$ verified from the oxygen uptake-work-rate relationship (8.9 ± 1.0 km·h^{-1}). Cycling power output for experimental trials was determined as 55% of MAP (163 ± 17 W), with MAP calculated as previously described (Hawley & Noakes, 1992).

Participants then completed a one-hour exertional-heat stress familiarization trial, at the running speed or power output used in experimental trials, in an environmental chamber at 35.2 ± 0.5°C ambient temperature (T_{amb}) and 22 ± 3% relative humidity (RH). Throughout the familiarization participants drank water *ad libitum*, and completed psychophysical

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measures including Rating of Perceived Exertion (RPE) on a 6-20 Borg Scale (Borg, 1982), thermal comfort rating (TCR; 13-point Likert-type thermal rating, with 7 indicative of comfortable, 10 indicative of hot, and 13 indicative of unbearably hot; adapted from Hollies and Goldman (1977)), and ratings of thirst and gastrointestinal symptoms (GIS) using a modified visual analogue scale (Gaskell et al., 2019). Nude body mass and water bottle mass were recorded before and after the familiarization to determine whole body sweat rate, which was subsequently used to estimate fluid requirements for participants during the experimental trials.

Experimental Procedure

Participants were provided with 2 L urine collection containers and asked to collect all urine produced in the three days preceding each experimental trial, excluding the first void on the first collection day. The first void on the morning of the experimental trial was collected in a separate bottle to allow separate analysis of urine specific gravity (USG). Participants were instructed to refrain from activities that caused significant perspiration during the urine collection period, to prevent sodium losses through thermoregulatory sweating. Prior to the low sodium diet (LNa) and high sodium diet (HNa) experimental visits, participants were provided with 3 d pre-prepared food (175 $kJ \cdot kg^{-1} \cdot day^{-1}$, protein: 1.5 $g \cdot kg^{-1} \cdot day^{-1}$, CHO: 6 $g \cdot kg^{-1} \cdot day^{-1}$, Na: 15 $mg \cdot kg^{-1} \cdot day^{-1}$), as well as methylcellulose capsules providing either 85 $mg \cdot kg^{-1}$ pharmaceutical grade NaCl (350 mg sodium per capsule) or placebo (caster sugar) in a randomized order. Capsules were consumed with main meals and snacks, and distributed evenly across the day, resulting in total dietary sodium intake of approximately 15 $mg \cdot kg^{-1} \cdot day^{-1}$ (LNa) or 100 $mg \cdot kg^{-1} \cdot day^{-1}$ (HNa). The LNa condition was chosen to reflect a sodium-restricted diet, and the HNa condition to reflect a sodium intake that was:

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a) greater than the typical population sodium intake (Land et al., 2018) by the same magnitude that LNa was reduced, and b) realistically achievable through conscious sodium loading by endurance athletes in the days preceding exercise (McCubbin et al., 2018). Participants and researchers interacting with participants were blinded to the content of capsules consumed before each experimental trial. Participants also completed an initial experimental trial, consuming their usual free-living diet (UDiet) whilst collecting all urine produced as described above. All other aspects of the experimental procedure were identical to HNa and LNa trials. The UDiet data has been included to allow comparison between UDiet and both increased and reduced sodium intakes.

Experimental visits were separated by one to two weeks, as previous work suggests that sodium balance stabilizes following abrupt changes in dietary intake within this timeframe (Conn & Arbor, 1963). Participants arrived at the laboratory fasted between 0830 and 0930, but the time consistent for the same participant. They were instructed to consume 8 $mL \cdot kg^{-1}$ water two hours prior to arrival to ensure euhydration before exercise commencement. Upon arrival, they were immediately provided breakfast (CHO: 1.5 $g \cdot kg^{-1}$, protein: 0.25 $g \cdot kg^{-1}$, fluid: 250 mL, Na: 420 mg). Thirty minutes later, after bladder voiding, total body water (TBW) was measured using multi-frequency bioelectric impedance analysis (Seca 515 MBCA; Seca Group, Hamburg, Germany) and corrected by regression equation, as previously validated against deuterium dilution in endurance athletes with a constant error of 0.02 L. Forty-five minutes after consuming breakfast, and after sitting for five minutes, blood was collected by venepuncture from the antecubital vein in a vacutainer (6 mL, 1.5 $IU \cdot mL^{-1}$ heparin), in a seated position. To monitor rectal temperature (T_{re}) during exercise, participants inserted a thermocouple 12 cm beyond the external anal sphincter (Grant REC soft insertion probe thermocouple; Grant 2010 Squirrel data logger, Shepreth, UK).

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The experimental protocol consisted of 2 h running or cycling at 55% of $\dot{V}O_{2max}$ (running) or 55% MAP (cycling), within an environmental chamber at 35.1 ± 0.6°C T_{amb}, 21.8 ± 1.5% RH and fan airspeed ~10.6 km·h^{-1} (running) or ~19.5 km·h^{-1} (cycling). Participants consumed water (approximately 23°C) of the same quantity in both trials, intended to limit body mass loss to 1.5%, based on sweat rate calculated during the familiarization. The required water volume was provided as four boluses, one given at the beginning of each 30 minute period, and participants instructed to consume the water evenly throughout this time. T_{re} was recorded every 5 min throughout exercise, and RPE, thermal comfort, perceived thirst and GIS every 10 min. Following completion of the 1st h of exercise, participants ceased exercising, and left the environmental chamber for five minutes to apply a second set of sweat patches (description below). A second blood sample was collected immediately post-exercise, as previously described within.

Sweat Sample Collection

Sweat samples were collected using the regional patch technique (Baker et al., 2009). Participants completed an 8-10 min warm up at the same intensity and ambient conditions as the experimental trial, which allowed the onset of sweat production, and reduced the risk of sample contamination from minerals in the sweat pore (Baker, 2017). Five sterile patches (Tegaderm+Pad, 3M Health Care, Minnesota, USA) were pre-weighed (Quintix 313-1S, Sartorius, Goettingen, Germany), then applied to the forehead (FH), right superior scapula (SS), upper chest (5 cm below the mid-point of the clavicle, UC), posterior mid-forearm (FA) and mid-thigh (MT) sites, as previously reported (Baker et al., 2009). Prior to application, each site was cleaned with an alcohol wipe, rinsed with deionized water, and dried with a clean laboratory wipe (Kimberly-Clark, Irving, TX, USA). Patches were

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removed with steel forceps that were pre-rinsed with deionized water and dried with clean laboratory wipes, when approximately 25% of the patch was visibly soaked with sweat, to prevent altered sample composition due to hidromeiosis (Baker, 2017). Exercise time was stopped during patch removal (approximately 30 seconds) to ensure the full 2 h of exercise was completed. Removed sweat patches were placed in pre-weighed glass petri dishes that had been rinsed in deionized water and air-dried. Local sweat rate (LSR) at each site was calculated from the change in patch mass before to after application, as previously reported (Smith & Havenith, 2011). Following removal and weighing, patches were immediately transferred to airtight plastic tubes (Salivette, Sarstedt, Nümbrecht, Germany) and centrifuged at 4,000 RPM and 4^{o}C for 10 min to extract sweat.

Sweat and Urine Analysis

Sweat and urine [Na^+] was determined by ion selective electrode (ISE) (LAQUATwin, Horiba, Kyoto, Japan), previously validated against ion chromatography for both sweat (Baker et al., 2014) and urine (Goulet & Asselin, 2015) samples. Two-point calibration was undertaken as per manufacturer's instructions. For calibration and measurement of sweat samples, the ISE surface was covered in a dry, pre-cut piece of laboratory wipe, and 45 μL samples pipetted onto the wipe. This technique compared to manufacturer-supplied sampling sheets with a coefficient of variation (CV) of 1.3%. For urine samples, 400 μL was pipetted directly onto the ISE surface following calibration with the same volume. The ISE surface was thoroughly washed with deionized water and dried with a clean laboratory wipe between each measurement. Urinary Na excretion (UNa) was calculated as the product of the urine [Na^+] and volume in each container. For LNa and HNa trials, only UNa data from the final two days of collection was analysed, due to a period of renal adjustment to

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the altered sodium intake on the first day of collection. Sweat [Na^+] was reported as individual patch site values and estimates of whole body sweat [Na^+], calculated using the regression equation developed by Baker et al. (2009) that incorporates data from all five sites (r= 0.97, ICC= 0.70).

Blood Analysis

Whole-blood hemoglobin (Hb) (Hb201+, Hemocue AB, Ängelholm, Sweden), and hematocrit (HCT) (centrifuged capillary tubes, Propper, Long Island City, USA) were used to calculate changes in plasma volume (P_v) relative to baseline, and to correct plasma variables (Dill & Costill, 1974). Remaining blood samples were centrifuged at 4000 RPM and 4^{o}C for 10 min, within 15 min of collection. Plasma was aliquoted into 1.7 mL micro-storage tubes and frozen at –80 °C until analysis, except for 100 μL (2 x 50 μL) that was used to determine plasma osmolality (P_{Osm}), in duplicate (CV 0.8%), by freeze-point osmometry (Osmomat 030; Gonotec, Berlin, Germany). Plasma [Na^+] was determined using ion selective electrodes (Cobas c 501, Roche Diagnostics, Risch-Rotkreuz, Switzerland) and analysed by local pathology services (Cabrini Pathology, Malvern, Victoria, Australia). Plasma aldosterone (DE5298; Demeditec Diagnostics GmbH, Kiel, Germany) and cortisol (RE52061; IBL International, Hamburg, Germany) were determined by ELISA. All variables were analysed as per manufacturer's instructions on the same day, with standards and controls on each plate, and each participant's samples on the same plate. Aldosterone and cortisol CV's were 3.9% and 5.8%, respectively.

Calculation of sweat sodium secretion and reabsorption rate

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To examine the contribution of indirect factors (plasma [Na^+], sweat production rate) and direct factors (regulated Na^+ reabsorption in the sweat gland) that contribute to regulation of sweat [Na^+] as a result of altered dietary sodium intake, and across exercise time period, calculations of sweat Na^+ secretion and reabsorption rates were performed using the method developed by Sato (1977), and utilised by Buono et al. (2008):

Na^+ secretion rate ($nmol \cdot cm^{-2} \cdot min^{-1}$) = LSR·plasma [$Na^+$]

Na^+ reabsorption rate ($nmol \cdot cm^{-2} \cdot min^{-1}$) = [(LSR·plasma [$Na^+$]) – (LSR·sweat [$Na^+$])]

Statistical Analysis

Using published standard deviations for sweat [Na^+] at regional patch sites (Dziedzic et al., 2014) and standard alpha of 0.05 and beta 0.85, it was calculated (G*Power v3.1.9.2, Universität Düsseldorf, Germany) that n= 9 would be required to provide adequate statistical power to detect a change of 25% in sweat [Na^+], a magnitude of change consistent with existing literature comparing 14-days usual and low sodium intakes (Hargreaves, et al., 1989; Yamazaki et al., 1994). Data are presented as mean and 95% confidence interval (CI), or mean ± SD, as indicated. The means of single time point data were analyzed using repeated measures ANOVA across the three dietary conditions. Data with multiple timepoints were analyzed using two-way repeated measures ANOVA to determine main effects of trial and time, and trial x time interactions, followed by Tukey's HSD post hoc analysis for pairwise comparisons, where applicable. To determine the contributing role of plasma variables (plasma [Na^+], P_{Osm} or P_v) and well as hormonal regulation of sweat gland function (plasma aldosterone and cortisol concentrations), Pearson correlation coefficients were calculated between individual variables and estimated whole body sweat [Na^+].

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Analysis was performed using SPSS 25.0 (IBM Corp., Armonk, New York, USA) with significance accepted at $p \leq 0.05$. There were no significant differences for control or outcome variables between cyclists and runners, therefore data was combined for the purpose of analysis and reporting. Blood samples could either not be collected or adequately analyzed for one participant in any trial and for three participants post-exercise in one trial, due to difficulties with venipuncture or insufficient sample volume, and therefore these data were excluded from comparative analysis. Sweat samples at FH could not be obtained in one participant and MT in another due to very low LSR, and therefore these data were also excluded from comparative analysis.

Results

Pre-exercise sodium and hydration status

All participants reported that they consumed 100% of the food and NaCl capsules provided during both LNa and HNa trials. One participant reported vomiting after ingestion of NaCl capsules at one meal, when NaCl capsules were not taken as instructed. There were no other reports of vomiting or severe nausea. UNa from the three days of UDiet was 46 (37-56) $mg \cdot kg^{-1} \cdot day^{-1}$, similar to the mid-point between LNa and HNa trials (47.5 $mg \cdot kg^{-1} \cdot day^{-1}$). UNa was significantly greater ($p < 0.001$) following HNa and lower following LNa ($p < 0.001$) compared to UDiet, and reflected the intended dietary Na intakes (Table 1). Pre-exercise nude body mass was lower following LNa compared to both UDiet ($p < 0.05$) and HNa ($p<0.01$). TBW was lower following LNa compared to UDiet and HNa ($p < 0.05$). Compared to UDiet, pre-exercise P_v was 1.8% lower following LNa, and 2.7% higher following HNa. However, this effect was highly variable and differences did not reach

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statistical significance (Table 1). First-void USG was lower ($p < 0.05$) on the morning of exercise for LNa compared to both UDiet and HNa, likely as a function of the reduced UNa.

Physiological markers, thirst and gastrointestinal symptoms during exercise

Body mass change, whole body sweat rate, water intake and P_v change during exercise are presented in Table 2. During all trials, P_v reduced as expected from the prescribed water intake. However, the P_v reduction was less during HNa compared to both LNa and UDiet ($p < 0.05$). There was no main effect of trial ($p = 0.273$), time ($p = 0.569$) or time × trial interaction ($p = 0.424$) for plasma [Na^+]. A main effect of trial was present for P_{Osm}, which was significantly lower following LNa compared to UDiet ($p < 0.05$), but no effect of time, or trial × time interaction, was observed. No main effects were observed for perceived thirst. A main effect of trial ($p = 0.044$) and time ($p < 0.001$) was observed for T_{re}, as well as a time × trial interaction ($p < 0.001$). As a simple main effect, T_{re} was greater in LNa compared to HNa ($p = 0.012$) but not UDiet ($p = 0.236$), or between UDiet and HNa ($p = 0.214$). The increase in T_{re} from the onset of exercise occurred more rapidly in LNa than UDiet and HNa, such that pairwise differences appeared from 40-70 min ($p < 0.05$). A noticeable reduction in T_{re} occurred from 60-70 min, due to exercise cessation required for sweat patch application. Thereafter T_{re} reached somewhat of a steady state during the 2nd h of exercise in all trials, with no pairwise differences from 80 min onwards (Figure 2a). There was a main effect of time observed for HR ($p < 0.001$), which was elevated compared to 10 min at all subsequent time points ($p < 0.05$) except for 70 min. Although visual inspection of the data suggested reduced HR with increasing sodium intake (Figure 2b), the main effect of trial was not statistically significant ($p = 0.124$), nor was a time × trial interaction observed ($p = 0.388$). A main effect of time ($p = 0.001$), but not trial ($p = 0.938$) or interaction ($p = 0.311$),

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was also observed for RPE, which increased with exercise duration (Figure 2c). A main effect of both trial ($p = 0.011$) and time ($p = 0.031$) was observed for TCR, with TCR generally increasing with exercise duration, and lower (more comfortable) during HNa at some but not all timepoints (Figure 2d).

A main effect of trial ($p = 0.037$) was observed for plasma cortisol concentration, with cortisol significantly greater in UDiet compared to HNa ($p = 0.006$) but not LNa ($p = 0.105$). No effect of time ($p = 0.115$) or interaction ($p = 0.111$) was observed. A main effect of trial ($p = 0.001$) and time ($p < 0.001$) was observed for plasma aldosterone (Table 3), but no interaction ($p = 0.188$). Simple main effects demonstrated aldosterone to be lower in UDiet ($p = 0.017$) and HNa ($p = 0.002$) compared to LNa, but not different between UDiet and HNa ($p = 0.190$). Incidence of any reported GIS was 27% following LNa, 40% following UNa and 20% following HNa. No effect of trial was observed for total GIS ($p = 0.166$), upper GIS ($p = 0.125$), lower GIS ($p = 0.482$), nausea ($p = 0.412$) or total gut discomfort ($p = 0.114$).

Sweat sodium concentration and local sweat rate

A main effect of time was observed for all patch sites ($p \leq 0.01$), whereby sweat [Na^+] was greater in the 2nd h of exercise compared to the 1st h (Figure 3a). A main effect of trial was also observed ($p < 0.05$ at all patch sites and estimated whole body sweat [Na^+]), with sweat [Na^+] increasing in proportion to sodium intake across the three trials. For whole body sweat [Na^+], the difference between UDiet and LNa, and UDiet and HNa, was similar (1st h (mean ± SD): LNa 36 ± 13 mmol·L^{-1}, UDiet 41 ± 12 mmol·L^{-1}, HNa 47 ± 15 mmol·L^{-1}; 2nd h: LNa 44 ± 15 mmol·L^{-1}, UDiet 49 ± 13 mmol·L^{-1}, HNa 55 ± 17 mmol·L^{-1} ; $p < 0.001$).

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A main effect of time was observed for LSR at FH ($p= 0.045$), UC ($p = 0.020$) and FA ($p= 0.025$) patch sites. Simple main effects demonstrated LSR to be greater in the 2nd h of exercise at these sites ($p< 0.05$), although pairwise comparisons did not consistently demonstrate statistically significant differences (Figure 3b). A main effect of trial was observed for LSR at the FH patch site only ($p= 0.026$), with LSR greater in HNa compared to LNa during the 2nd h ($p= 0.014$).

Sweat sodium secretion and reabsorption rates

No main effect of trial, time or time × trial interaction was observed for sweat Na^+ secretion rate at any sweat patch site (Figure 3c). A main effect of trial was observed for sweat Na^+ reabsorption at the UC ($p= 0.039$), FA ($p= 0.027$) and MT ($p= 0.040$) sites, with Na^+ reabsorption increased following LNa compared to HNa ($p< 0.05$ at all three sites) but not UDiet. There was no main effect of time or time × trial interaction for sweat Na^+ reabsorption at any site. Pairwise differences are shown in Figure 3d.

Correlation of variables with sweat [Na^+]and sodium losses

The change in UNa between UDiet and LNa, and between UNa and HNa, was not correlated with the subsequent change in WB sweat [Na^+] (UNa *vs* LNa: $r= 0.21$, $p= 0.470$; UNa *vs* HNa: $r= 0.293$, $p= 0.310$). The correlation between variables implicated in sweat gland function or output (pre-exercise plasma [Na^+], aldosterone, cortisol, P_{Osm}, and change in pre-exercise P_v between trials), and both the estimated whole body sweat [Na^+] and sweat sodium losses across all three trials, are presented in Table 4. Only pre-exercise plasma

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aldosterone (1st h r= -0.568, p= 0.027; 2nd h r= -0.675, p= 0.006) was correlated with sweat [Na^+], and no variable was correlated with sweat sodium losses.

Discussion

The aims of the present study were to investigate the impact of 3 days high (HNa) and low (LNa) dietary sodium intake, in comparison to each other and to usual habitual diet (UDiet), on aspects of sodium balance before and during exercise; as well as the effect on cardiovascular, thermoregulatory, and gastrointestinal variables. As hypothesized, UNa increased progressively from LNa to HNa, and was reflected in pre-exercise plasma aldosterone concentration differences. Increasing sodium intake tended to result in greater pre-exercise nude body mass, P_v and P_{Osm}, but not plasma [Na^+], although these differences were smaller and less consistent when comparing HNa to UDiet. During exercise, a smaller reduction in P_v was observed following HNa compared to UDiet and LNa, and the rate of rise in T_{re} was attenuated, although final T_{re} was not different between trials, and differences in HR between trials failed to reach statistical significance. Changes in plasma aldosterone, cortisol, P_{Osm} and plasma [Na^+] from pre- to post-exercise were not affected by trial, nor was GIS. In accordance with our hypothesis, the main finding was a clear effect of dietary sodium intake on sweat [Na^+], with LNa resulting in a reduction in estimated whole body sweat [Na^+] of 10-11%, and HNa an increase of 10-12%, compared to UDiet. To the best of our knowledge, the present study is the first to provide competitive recreational endurance athletes with a controlled dietary sodium intake, blinded and proportional to body mass, over a timeframe (3-days) that reflects the period of altered sodium ingestion before competition (McCubbin et al., 2018). Collecting sweat samples using previously reported patch sites allowed estimation of whole body sweat [Na^+] from established regression equations (Baker et al., 2009), showing that in response to dietary sodium intake, altered

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463 sweat Na^+ reabsorption, rather than secretion, was the most likely reason for the observed
464 difference between trials.

465 Considering the multifactorial nature of sweat [Na^+] regulation, several potential
466 mechanisms could potentially contribute to the differences observed between dietary
467 sodium intakes, including differences in plasma [Na^+], P_{Osm} or P_v, as well as changes in
468 hormonal regulation of sweat gland reabsorptive function. The present study observed
469 minimal differences in pre-exercise plasma [Na^+] between interventions, although P_{Osm} and
470 P_v were both greater following UDiet and HNa compared to LNa. Previous research has
471 shown that increased P_{Osm} can increase the T_{re} threshold for the onset of sweating, and lower
472 the sweat rate itself, independent of P_v in both passive (Takamata et al., 1995, 2001) and
473 exercise-based (Sawka et al., 1985; Fortney et al., 1984) models of heat stress. Altered sweat
474 rate could then influence sweat Na^+ secretion, and therefore sweat [Na^+] (Buono et al.,
475 2008). However, in the current study, as in others specifically investigating the effect of
476 sodium intake on sweat sodium losses (Armstrong et al., 1985; Hargreaves et al., 1989;
477 Koenders et al., 2017; Konikoff et al., 1986), differences in whole body and local sweat
478 rates were not observed between trials despite differences in P_v and P_{Osm}, nor was plasma
479 [Na^+] affected by dietary sodium intake.

480 In contrast, differences in pre-exercise aldosterone and cortisol concentration have been
481 suggested to play a role in regulating sweat Na^+ reabsorption, through expression of ion
482 channels on the luminal surface of the gland (Sato et al., 1989; Castro-Sepulveda et al.,
483 2019). In the present study, the concentration of plasma aldosterone was lower, and plasma
484 cortisol higher, following three days LNa compared to UDiet and HNa. However, only
485 plasma aldosterone concentration was correlated with sweat [Na^+]. The correlation between
486 pre-exercise aldosterone and sweat [Na^+] is in accordance with previous research (Yoshida

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et al., 2006). Supporting a causative role of aldosterone on sweat Na^+ reabsorption, intervention studies that have administered local or systemic exogenous aldosterone, have reported significant reductions in sweat [Na^+]; albeit at least 6 h after administration (Sato & Dobson, 1970; Collins, 1966).

The results from the present study also raise the question as to the mechanism underlying differences in pre-exercise P_{Osm} between trials. P_{Osm} can be predicted from plasma concentrations of sodium, potassium, glucose and urea (Hooper et al, 2015). No differences between trials were observed for pre-exercise plasma [Na^+] (Table 3), potassium or glucose (data not shown), leaving urea the likely contributor to P_{Osm} differences. Although urea was not measured in the current study, previous work suggests that urea plays a key role in the renal regulation of sodium balance, with increased urea production a response to increasing sodium intake, as it facilitates increased water reabsorption in the renal medulla, preventing a significant diuresis from accompanying the upregulated natriuresis (Rakova et al., 2017).

The effect of dietary sodium intake in the days preceding exercise on thermoregulatory and cardiovascular variables during the exercise bout is also of relevance to athletes, particularly when exercising at high intensities and in hot ambient conditions. The observed pattern of change in T_{re} in particular is of interest. Whilst all trials reached a similar T_{re} at the conclusion of exercise, the initial rise in T_{re} was more rapid in the LNa trial. There are two potential explanations for this finding. Firstly, the increased pre-exercise TBW in UDiet and HNa trials would require a greater degree of energy expenditure in order to raise core body temperature to the same extent (i.e., increasing exercise duration, as seen in HNa and UDiet compared to LNa). Secondly, the difference in measured T_{re} may also be at least in part due to differences in blood flow to the rectum, which can be altered by pre-exercise P_v (Taylor et al., 2014). Regardless of the mechanism, exercise-induced changes in T_{re} were small in

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511 the present study, since mean T_{re} did not exceed 38.5°C in any trial or timepoint, and
512 appeared to reach a steady state in the 2nd h of exercise, a pattern consistent with similar
513 exercise protocols (Costa et al 2014; Gill et al., 2016; Alcock et al 2018; Snipe et al 2017;
514 Snipe et al 2018a; 2018b). A lack of clear effect of pre-exercise sodium intake on T_{re} is also
515 consistent with previously published data from similar intensity steady state exercise
516 (Hamouti et al., 2014), and for sodium ingestion during exercise when water intake is fixed
517 (Earhart et al., 2014). In contrast, the effect of sodium-influenced differences in pre-exercise
518 TBW and P_v are more likely to be evident during higher intensity exercise, where the rate
519 of heat production is greater, and thermoregulation is likely to become a limiting factor to
520 performance (Racinais et al., 2018). Previous studies employing an acute sodium loading
521 strategy (20-40 $mg \cdot kg^{-1}$, 1-2 h prior to exercise) increased pre-exercise P_v, reduced the rate
522 of rise in HR and T_{re} during a time to exhaustion test at 70% $\dot{V}O_{2peak}$, the performance of
523 which was improved as a result (Sims et al., 2007a,b). Even following 2 h of moderate
524 intensity steady state exercise, acute pre-exercise sodium loading resulted in improved time
525 trial performance of approximately 10 min duration (Hamouti et al., 2014). Whilst
526 differences in HR and T_{re} were minimal even at the completion of the time trial, differences
527 were apparent in stroke volume, and therefore cardiac output (Hamouti et al., 2014). It
528 would appear that the relevance of pre-exercise sodium intake for athletes may depend on
529 the specific demands of their sport, with shorter, higher intensity endurance events (e.g.
530 marathon or shorter distance running events, and Olympic distance triathlon), or longer
531 events with interspersed high intensity efforts (e.g. road cycling) most likely to benefit from
532 a higher sodium intake, especially when gastrointestinal tolerance or opportunities to drink
533 during exercise are limited.

534 The current finding of a 23-28% difference in estimated whole body sweat [Na^+] between
535 LNa and HNa, and 10-12% between UDiet and both LNa and HNa, is in contrast to some

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of the previous studies on the topic, recently summarized in a systematic review (McCubbin & Costa, 2018). Possible explanations for this outcome include: firstly, the current study compared sodium intakes that were both substantially lower and higher than typical intakes (i.e. 15 $mg \cdot kg^{-1} \cdot day^{-1}$ and 100 $mg \cdot kg^{-1} \cdot day^{-1}$), and a greater difference in sodium intake compared to many other studies. Secondly, the current study utilized the regional patch technique for sweat collection rather than the whole body washdown (WBW) method, which is considered the reference method for obtaining samples during exercise (Baker et al., 2018). The reasons for using the regional patch technique were that the WBW method is unsuitable for treadmill running, because shoes cannot be worn during this technique, and both the participant and equipment must be thoroughly rinsed with several liters of solution following the exercise period, a technique clearly unsuitable for a motorized treadmill (Shirreffs & Maughan, 1997). The regional patch method with five sites allowed us to observe if the change in sweat $[Na^+]$ occurred universally across body sites, which was found to be the case. In addition, the use of the regional patch method allowed the investigation of the effects of the intervention on sweat gland function, providing further insight into the mechanisms underlying the altered sweat $[Na^+]$ that has not been reported in dietary intervention studies to date.

Two additional practical implications for athletes have emerged from these findings. Firstly, increasing sodium intake substantially in the days preceding exercise had minimal impact on both absolute plasma $[Na^+]$, and the change in plasma $[Na^+]$ during exercise, when only water was consumed. However, whilst a deliberate increase in sodium intake in the days preceding exercise increased P_v and may offer a potential thermoregulatory benefit, the increased sweat $[Na^+]$ in the HNa condition would theoretically increase the sodium intake requirement during exercise, in order to maintain plasma $[Na^+]$. Future research is warranted to better understand these potential trade-offs. Secondly, for athletes undergoing sweat

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composition testing in training, to inform expected sodium losses during competition, any difference in dietary sodium intake between these timepoints is likely to result in only a modestly inaccurate estimation of sweat sodium losses. In fact, such differences (i.e. less than 12% between UDiet and HNa in the present study), fall within the typical day-to-day variability observed in previous reliability studies of sweat [Na^+] testing (Baker, 2017); albeit dietary sodium intake was not controlled in such studies. The observed differences in sweat [Na^+] in the current study may still be of relevance to researchers during laboratory studies of sodium replacement during exercise, in that careful control of dietary sodium intake in the days preceding exercise would ensure more predictable sweat sodium excretion (and therefore replacement) occurs.

There are some limitations with the current study. Firstly, the method of sweat sample collection, using the regional patch method rather than WBW as the reference method, has already been discussed. Secondly, the application of both running and cycling in the current study was a deliberate choice, to identify any differences between exercise modes on sweat losses, cardiovascular and thermoregulatory variables. Whilst the authors acknowledge this as a potential limitation, these variables were found not to be different between exercise modalities, and therefore data was combined for presentation purposes. Finally, the use of free-living diet (UDiet) as a comparator to both LNa and HNa means that nutrient intake during UDiet was not standardized between participants, or between UDiet and the other dietary conditions in the study. This difference could potentially influence pre-exercise TBW and during-exercise RPE with UDiet compared to LNa and HNa, due to differences in muscle glycogen content and associated intracellular water (Olsson & Saltin, 1970). Caution should be made therefore in interpreting differences in TBW between UDiet and the other conditions. It was noted that RPE was not different between any of the conditions in the study, including the highly controlled LNa and HNa conditions, suggesting that any

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effect of muscle glycogen content is unlikely influence interpretation of RPE. UNa resulting from UDiet was also more variable between participants than LNa and HNa due to the lack of control of dietary sodium intake. However, the magnitude of increase or decrease in UNa between UDiet and both LNa and HNa did not correlate with the magnitude of change in sweat [Na^+], suggesting that the homeostatic response to changes in sodium intake also vary between individuals.

Conclusion

Three days of a high sodium diet (100 $mg \cdot kg^{-1} \cdot day^{-1}$) prior to steady state endurance exercise in the heat resulted in a reduced rate of rise in T_{re} compared to three days of low sodium diet (15 $mg \cdot kg^{-1} \cdot day^{-1}$). However, differences between usual and high sodium intakes were minimal despite increased total body water and plasma volume from the high sodium diet. In addition, sweat [Na^+] was reduced when restricting dietary sodium intake, and increased when sodium intake was increased from usual levels, and these changes correlated with pre-exercise plasma aldosterone. Future research should aim to assess the practical significance of these physiological changes on exercise performance in hot ambient conditions, across a range of exercise modalities, intensities and durations relevant to competitive athletes.

Competing Interests

The authors have no conflicts of interest, financial or otherwise, to declare.

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616

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Table 1. Effect of three days of dietary sodium intake on urinary sodium excretion, and markers of pre-exercise hydration status.

	Low Na	*Usual Na*	*High Na*	*p value*
Urinary Na excretion (mg·day^{-1})	1171 (932-1410) ^{aabb}	3517 (2823-4210) ^{bbcc}	6091 (5561-6621) ^{aacc}	< 0.001
(mg·kg^{-1}·day^{-1})	16 (12-19) ^{aabb}	46 (37-56) ^{bbcc}	79 (73-86) ^{aacc}	< 0.001
Pre-exercise nude body mass (kg)	75.9 (73.2-78.5)	76.5 (73.8-79.2) ^{c}	76.7 (74.0-79.5) ^{cc}	< 0.001
Total body water (L)	44.1 (42.7-45.5) ^{b}	44.1 (42.8-45.4) ^{b}	44.8 (43.1-46.5) ^{ac}	0.008
Urine specific gravity	1.015 (1.012-1.018) ^{a}	1.018 (1.013-1.023)	1.017 (1.014-1.020)	0.032
Plasma Volume Change (% relative to Usual Na)	-1.8 (-6.7 to 3.2)	NA	2.7 (-2.8 to 8.1)	0.097

Mean (95% CI): ^{a} p< 0.05 and ^{aa} p< 0.01 *vs* UNa, ^{b} p< 0.05 and ^{bb} p< 0.01 *vs* HNa, ^{c} p< 0.05 and ^{cc} p< 0.01 *vs* LNa.

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Table 2. Fluid balance and plasma volume change during 2 h running (55% $\dot{V}O_{2max}$) and cycling (55% MAP).

	Low Na	*Usual Na*	*High Na*	*p value*
Whole body sweat rate ($mL \cdot h^{-1}$)	1245 (1077-1413)	1270 (1132-1408)	1235 (1092-1377)	0.684
Water intake ($mL \cdot h^{-1}$)	629 (530-728)	627 (496-757)	621 (512-730)	0.987
Body mass loss (%)	1.6 (1.2-2.0)	1.7 (1.2-2.1)	1.6 (1.2-1.9)	0.676
Plasma volume change (%)	-7.5 (-10.7 to -4.3) [a]	-7.1 (-8.8 to – 5.4) [a]	-2.5 (-6.3 to 1.3) [bc]	0.027

Mean (95% CI): [a] $p < 0.05$ *vs* HNa, [b] $p < 0.05$ *vs* UNa, [c] $p < 0.05$ *vs* LNa.

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Table 3. Changes in plasma aldosterone, cortisol, sodium and osmolality during 2 h running (55% $\dot{V}O_{2max}$) and cycling (55% MAP).

	Low Na		***Usual Na***		***High Na***		***Main effects***		
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Effect of time	Effect of trial	Time × Trial Interaction
Plasma aldosterone ($pg \cdot mL^{-1}$)	240 (193-286) ^{aab}	506 (415-597) ^{aabee}	170 (116-224) ^{c}	366 (313-419) ^{cee}	141 (111-171) ^{cc}	313 (206-421) ^{cce}	<0.001	0.001	0.188
Plasma cortisol ($nmol \cdot mL^{-1}$)	488 (417-560) ^{b}	634 (506-761)	595 (486-705) ^{c}	675 (539-812) ^{aa}	541 (444-638)	539 (439-639) ^{bb}	0.115	0.037	0.111
Plasma osmolality ($mOsm \cdot kg^{-1}$)	285 (273-297) ^{b}	284 (272-295) ^{b}	298 (295-300) ^{c}	298 (295-301) ^{c}	295 (289-300)	295 (290-299)	0.840	0.044	0.653
Plasma sodium ($mmol \cdot L^{-1}$)	140 (139-142)	141 (139-142)	141 (139-143)	140 (139-141)	141 (139-144)	141 (140-143)	0.569	0.273	0.424

Mean (95% CI): ^{a} p< 0.05 and ^{aa} p< 0.01 *vs* HNa, ^{b} p< 0.05 *vs* UNa, ^{c} p< 0.05 and ^{cc} p< 0.05 *vs* LNa, ^{e} p< 0.05 and ^{ee} p< 0.01 *vs* pre-exercise.

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Table 4. Correlations between plasma variables and estimated whole body sweat [Na^+] and sweat sodium losses

	Estimated whole body sweat [Na^+] ($mmol \cdot L^{-1}$)		***Sweat sodium losses ($mmol \cdot h^{-1}$)***	
	1st h	2nd h	1st h	2nd h
Pre-exercise plasma aldosterone ($pg \cdot mL^{-1}$)	-0.568 [aa]	-0.675 [aa]	-0.293	-0.400
Pre-exercise plasma cortisol ($nmol \cdot mL^{-1}$)	0.101	0.108	0.025	0.076
Pre-exercise plasma osmolality ($mOsm \cdot kg^{-1}$)	0.219	0.134	0.083	-0.009
Pre-exercise plasma sodium ($mmol \cdot L^{-1}$)	0.180	0.213	0.095	0.161
Pre-exercise plasma volume change from UDiet (%)	-0.236	-0.325	-0.314	-0.354

Values represent correlation coefficient (r): [aa] $p < 0.01$.

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Figure Captions

Fig. 1. Illustrative description of experimental procedures.

UDiet: Usual free-living diet (46 mg $\cdot kg^{-1} \cdot day^{-1}$). LNa: low sodium diet (15 mg $\cdot kg^{-1} \cdot day^{-1}$). HNa: high sodium diet (100 mg $\cdot kg^{-1} \cdot day^{-1}$). MAP: maximal aerobic power. T_{amb}: ambient temperature. RPE: rating of perceived exertion. HR: heart rate. GIS: gastrointestinal symptoms. T_{re}: rectal temperature. TBW: total body water. MF-BIA: multi-frequency bioelectric impedance analysis.

Fig 2. Rectal temperature (A), heart rate (B), rating of perceived exertion (C), and thermal comfort rating (D) in response to 2 h of running and cycling at 55% $\dot{V}O_{2max}$ or MPO in T_{amb} 35°C following HNa (black squares), UDiet (grey squares), or LNa (white squares). Mean ± SD: main effect of time $^{\#}$ $p< 0.05$ and $^{\#\#}$ $p< 0.01$, main effect of trial § $p< 0.05$, time × trial interaction ^ $p< 0.01$, ^{a} $p< 0.05$ and ^{aa} $p< 0.01$ *vs* 10 min, ^{b} $p< 0.05$ and ^{bb} $p< 0.01$ LNa *vs* HNa, ^{c} $p< 0.05$ LNa *vs* UDiet, ^{dd} $p< 0.01$ UDiet *vs* HNa.

Fig. 3. Sweat $[Na^+]$ (A), Local Sweat Rate (B), sweat sodium secretion (C) and reabsorption (D) rate during the 1st and 2nd h of exertional heat stress (running and cycling at 55% maximal oxygen uptake/maximal aerobic power in 35 °C T_{amb}) following HNa (black bars), UDiet (grey bars), or LNa (white bars). Mean ± SD: ^{a} $p< 0.05$ and ^{aa} $p< 0.01$ *vs* 1st h, ^{b} $p< 0.05$ and ^{bb} $p< 0.01$ *vs* LNa, ^{c} $p< 0.05$ *vs* UDiet.

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Fig. 1.

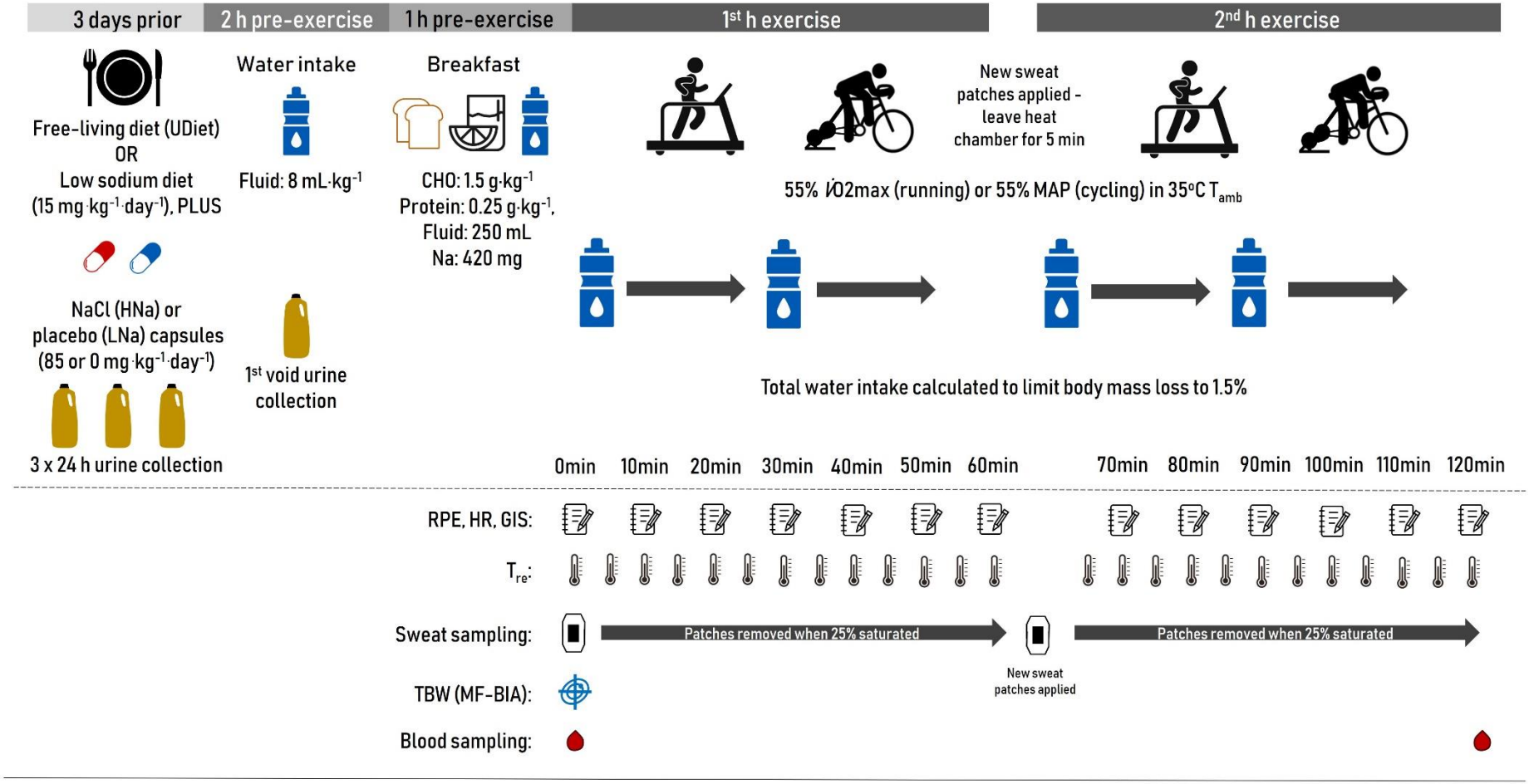

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Fig 2.

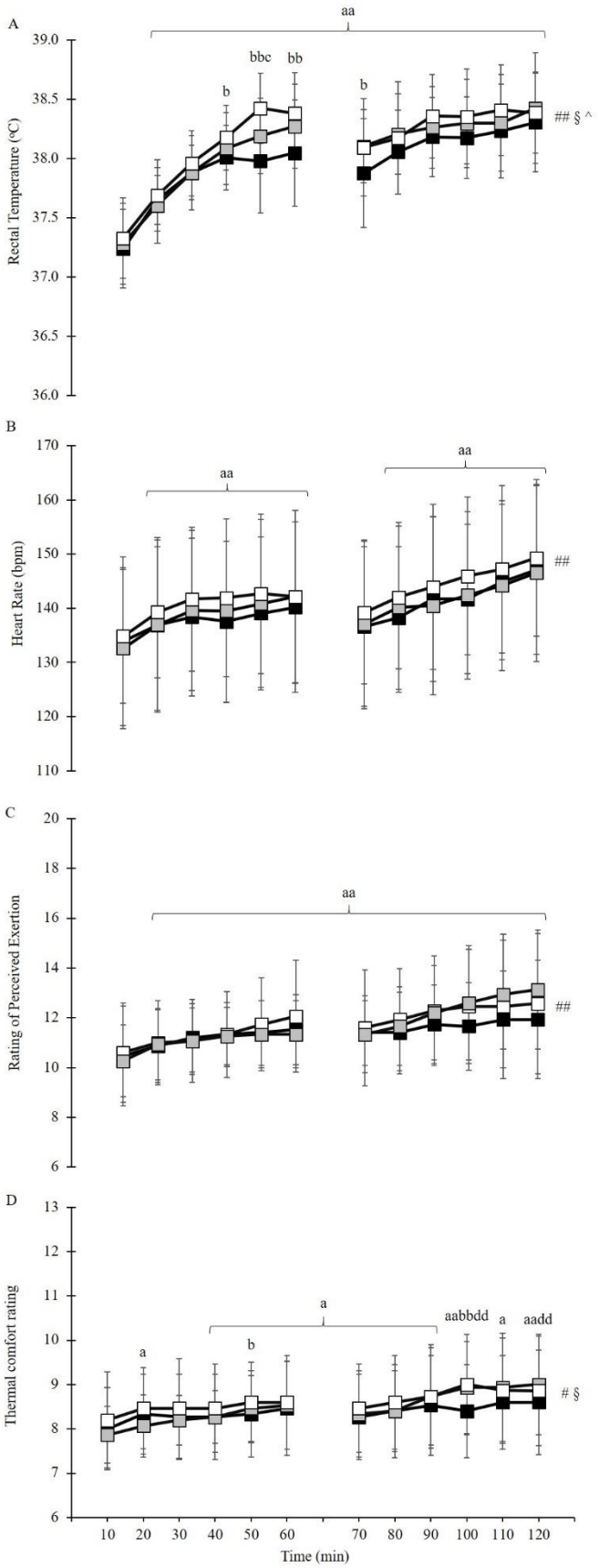

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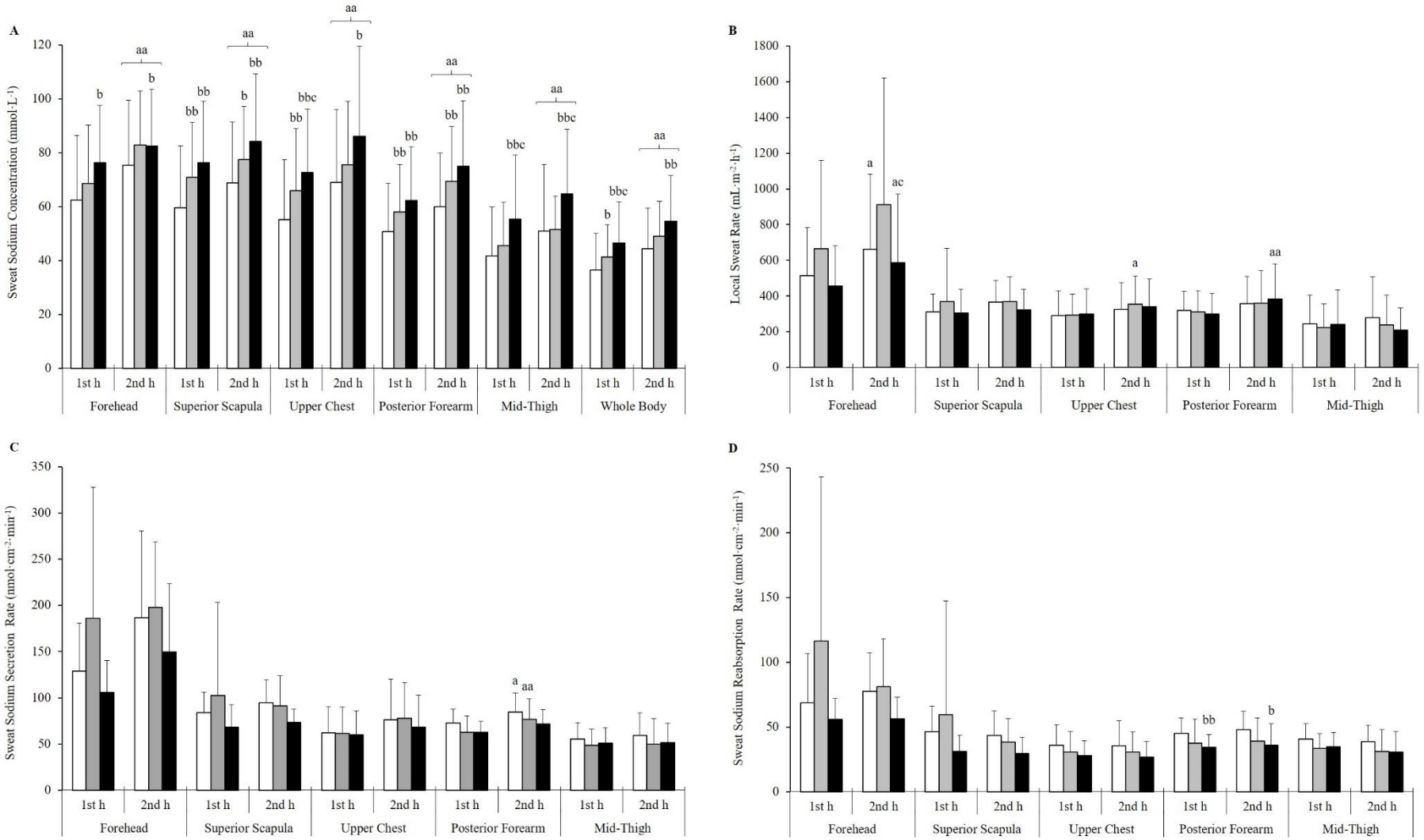
Fig 3.



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